IN THE SPECIFICATION

Replace paragraph **[0011]** with the following:

[0011] The isolate was specifically designed to utilize an important virulence factor for the initial development of columnaris disease, adhesion. Adhesion is proven as an essential preliminary step in a great number of human and animal diseases. It is well established that bacteria must first adhere to biological substrata prior to any infectious process (Ofek and Doyle, 1994, Bacterial Adhesion to Cells and Tissues, Chapman & Hall, Inc.). How bacteria adhere to various substrata is still not well understood, but it is thought to involve a complex association of factors that are under genetic control. Studies of bacteria with natural-occurring mutations have revealed a great deal about the mechanisms controlling adhesion in bacteria. Such mutations, while quite rare, do occur in such organisms as ~~*Escherichia*~~ *Escherichia coli*, an enteric pathogen of humans (Nowicki et al., 1985, FEMS Microbiol. Lett., 26:35-40). An *E. coli* bacterial cell which is incapable of adhering to human intestinal microphili is also incapable of proper replication and colonization, which are prerequisites to infection. *F.*

columnare is also thought to first adhere, then colonize and finally to infect fish tissue. Studies of whole-cell lysates of *F. columnare* have shown the importance of surface proteins for recognition of the bacteria by the immune system of channel catfish (Bader et al., 1998; Am. J. Vet. Research, 58, pp. 985-988). The pathogen is usually observed adhering to the skin of fish in large numbers prior to active infection (Plumb, 1994). β-lactams (a class of antibiotics), such as ampicillin are known to modify surface proteins and have been shown to affect adhesion. The premise of the invention was that the use of β-lactams (ampicillin) in the production of adhesion deficient bacterial mutants may be possible through modification of the ~~bacteriums's~~ bacterium's outer membrane proteins (OMP's). Since the modification of the OMP's may result in less adhesion, the *F. columnare* may be less virulent.

Replace paragraph **[0023]** with the following:

[0023] A plate count assay, using tissue from recently dead fish, was specifically designed for these studies to provide statistically valid quantitative measurements of

adhesion, tissue specificity and adhesion to tissue over time. Fifty four experimental fish were held, 18 fish per tank, and immersion exposed, with 3 treatments, 10^6 CFU/ml ARS-FC1-96, 10^6 CFU/ml NRRL B-30687, and MCM media without bacteria (negative control). Mutant ARS-MCFC-01 was not evaluated *in vivo* because it did not significantly differ from the parent in colony morphology, nor in the *in vitro* adhesion assay. Three fish per treatment tank were sampled at times 0, 0.5, 1, 2, 4, 8 h following challenge. Prior to sampling, each fish was pithed (an approved method for euthansia) with a sterile needle. After pithing, approximately 1 cm^2 of skin was aseptically removed from one side of each fish, weighed, on a ~~preweighed~~ pre-weighed plastic weight boat, and put in 2 mL of sterile MCM medium. Next, approximately 2 gill raker sections of gill were aseptically removed, and handled in a manner similar to the skin. The gill tissue was collected between 2-5 min after the skin. Both tissues were shaken on a radial shaker (15 min, 22°C), transferred to a 4 mL cryotube (Corning, Corning, New York) containing 2 mL of sterile MCM medium, and homogenized on ice (1 min) using a tissue homogenizer. Tissue homogenates were then serially diluted in sterile

MCM medium on a microtiter plate, 10^{-1}-10^{-4}. Dilutions (0.01 mL) were then streaked onto bacteriological plates containing sterile MCM medium, and incubated (28°C for 48 h). Colony forming units were counted following incubation period for each dilution. Total CFU/mL were then calculated by CFU/volume plated multiplied by the dilution factor and the result corrected for tissue weight differences by dividing CFU by sample weight (g). For statistical evaluation, "No growth" was considered to be ≤500 CFU/g.

Replace paragraph **[0024]** with the following:

[0024] Bacterial adhesion of NRRL B-30687 was evaluated using an *in vivo* adhesion assay and was found to have significantly ($P \leq 0.05$) less ability to adhere to skin tissue then ARS-FC1-96 and was the same as the negative control in ability to adhere to skin tissue throughout the 8 h test. Cumulative mean CFU/g for the skin tissue throughout the 8 h sampling period for each group (ARS-FC1-96, NRRL B-30687, and negative control) of 18 fish were 3694, 500, and 500, respectively. Bacterial adhesion of mutant NRRL B-30687 could adhere to the gill, but this

adhesion was significantly ($P \leq 0.05$) less ~~then~~ than ARS-FC1-96 and was significantly more than the negative control throughout the 8 h test. Cumulative mean CFU/g for the gill tissue ~~though out~~ through out the 8 h sampling period for each group (ARS-FC1-96, NRRL B-30687, and negative control) of 18 fish were 33333, 8389, and 500, respectively.